

Crane flies (Diptera: Tipuloidea: Tipulidae) from Dayaoshan National Nature Reserve, China and analysis of DNA barcodes, with description of one new species in the genus *Indotipula*

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Abstract: This paper reports six species of crane flies from Dayaoshan National Nature Reserve, Guangxi Zhuang Autonomous Region, China, including one new species, *Indotipula jinxiuensis* sp. nov. The males of *Pselliophora guangxiensis* Yang & Yang, 1988 and *Holorusia basiflava* Yang & Yang, 1993 and female of *Pselliophora xanthopimplina* Enderlein, 1921 are redescribed and illustrated with new morphological characters. The females of *P. guangxiensis* and *H. basiflava* are described and illustrated for the first time. A key for separating known species of *Indotipula* Edwards, 1931 from China is provided. DNA barcodes of all species in this study are provided and analyzed.

Key words: Nematocera; DNA barcodes; redescription; taxonomy; key

大瑶山国家自然保护区大蚊科种类记述及 DNA 条形码分析并记 *Indotipula* 属一新种（双翅目：大蚊总科）

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摘要: 记述采自大瑶山国家自然保护区的 6 种大蚊, 其中包括 1 新种: 金秀印大蚊 *Indotipula jinxiuensis* sp. nov.。对广西比栉大蚊 *Pselliophora guangxiensis* Yang & Yang, 1988 和基黄棘膝大蚊 *Holorusia basiflava* Yang & Yang, 1993 的雄虫以及拟蜂比栉大蚊 *P. xanthopimplina* Enderlein, 1921 的雌虫进行了重新描述并补充了新的形态特征。对广西比栉大蚊和基黄棘膝大蚊的雌虫进行了首次描述。同时提供了印大蚊属 *Indotipula* Edwards, 1931 中国物种的分种检索表。提供了全部物种的 DNA 条形码并对序列进行了分析。

关键词: 长角亚目; DNA 条形码; 重新描述; 分类; 检索表

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Introduction

Dayaoshan National Nature Reserve is located in Jinxiu County, Guangxi Zhuang Autonomous Region, restricted in the transition zone of the mid-subtropical area and the southern subtropical area. The special geological conditions and climates have formed this area resulting in high species diversity.

The fauna of crane flies in Dayaoshan National Nature Reserve has been relatively poorly documented. Only three new species were formerly reported in this area: *Pselliophora pallitibia* Yang & Yang, 1988, *Pselliophora jinxiuensis* Yang & Yang, 1988 and *Nephrotoma jinxiuensis* Yang & Yang, 1993 (Yang & Yang 1988, 1993a). From 2015 to 2016, we launched an investigation of crane flies species in this area. In the course of field collecting, one new species in the genus *Indotipula* and five known species were found.

Indotipula was established by Edwards (1931) with the type species *Tipula walkeri* Brunetti, 1911, from Southeast Asia by original designation. It was originally placed in the genus *Tipula* Linnaeus as a subgenus, but subsequently elevated to a generic level by Savchenko (1983). It is characterized by the following characters: nasus elongate; posterior tibia with two spurs which are unequal; squama naked; R_{1+2} entire, Rs moderate length, subequal to $m-cu$; male terminalia with tergite nine produced into bifid lobes apically bearing blackened spicules, outer gonostylus compressed, covered with abundant setae, inner gonostylus greatly modified; ovipositor long and slender (Alexander 1935). Up to now, 66 species and one subspecies have been reported worldwide and they are mainly restricted to the Oriental Region. The Chinese fauna of *Indotipula* is poorly represented with only three species and one subspecies, *Indotipula demarcata* (Brunetti, 1911), *Indotipula suensonii* (Alexander, 1925), *Indotipula yamata* (Alexander, 1914) and *Indotipula yamata subyamata* (Alexander, 1933) (Oosterbroek 2017). While sorting crane flies specimens collected from Dayaoshan National Nature Reserve, a new species of *Indotipula* was noticed. In this paper, the new species is described and illustrated, and a key is provided for separating all known species from China.

The females of *Pselliophora guangxiensis* Yang & Yang, 1988 and *Holorusia basiflava* Yang & Yang, 1993 are described and illustrated for the first time. The males of *Pselliophora guangxiensis* and *Holorusia basiflava* and the female of *Pselliophora xanthopimplina* Enderlein, 1921 are redescribed and illustrated adding new morphological characters. *Pselliophora xanthopimplina* and *Tipula (Formotipula) maolana* Li, Yang & Chen, 2013 are first reported from Guangxi Zhuang Autonomous Region. *Tipula (Formotipula) holoserica* (Matsumura, 1916) is first recorded from the China mainland. Data of DNA barcoding is provided for facilitating identification of the species in this area. Also, the genetic distances, variations of sites and nucleotide compositions of COI sequences for these six species are calculated and analyzed.

Material and methods

Taxonomic analysis

Photographs of the body parts of adults were obtained using a SOIFXTZ-E stereomicroscope (SOIF, Shanghai, China). The hypopygium of the male and the ovipositor of

the female were removed and macerated in 10% NaOH for one hour in a 50°C water bath, observed in glycerin and illustrated under a SOIFXTZ-E stereomicroscope (SOIF, Shanghai, China). The body length was measured from the vertex of the head to the tip of the genitalia. All measurements were made in millimeters (mm) with the aid of a digital caliper. The angles between compressor apodemes and posterior immovable apodemes of the sperm pump were measured by ImageJ software. The terminology and methods of description and illustration follow that of Alexander & Byers (1981) and Frommer (1963). The type specimens are deposited in the animal specimen room, School of Life Sciences, Anqing Normal University. Qiulei MEN was responsible for the taxonomic portion of this paper and thus is the author of the new species.

Abbreviations. AIA—anterior immovable apodeme; CA—compressor apodeme; PIA—posterior immovable apodeme; T—tergite; S—sternite; OG—outer gonostylus; IG—inner gonostylus; ep—ear-shaped process; hp—horn-shaped process; up—upper process; lp—lower process.

Molecular analysis

Genomic DNA was extracted from the leg muscle of a dry preserved specimen using Biomiga Insect gDNA Kit (Biomiga, USA). The partial sequence of the mitochondrial COI gene was amplified using the universal primers for metazoan invertebrates, LCO1490 (5'-GGTCAACAAATCATAAAGATATTG-3') and HCO2198 (5'-TAAACTTCAGGGTGA CCAAAAAAT-3') (Folmer *et al.* 1994). PCR amplifications were employed using a final volume of 20 μ l containing 10 μ l 2 \times Pfu PCR MasterMix (Tiangen, Beijing, China), 0.75 μ l each primer (10 μ M), 1 μ l DNA template and 7.5 μ l ddH₂O. PCR amplification was employed with denaturation at 95°C for 5 mins, followed by 45 cycles of 30 s at 95°C for denaturation, 30 s at 50°C for annealing and 1 min 30 s at 72°C for extension, with a final extension at 72°C for 10 mins. All PCR sets included a negative control reaction tube in which all reagents were included but the template DNA. After electrophoresis using 1% agarose gel, the target DNA was sequenced by Genescrypt Biotechnology Co., Ltd. (Nanjing, China). The partial COI sequences were aligned with CLUSTAL X (Thompson *et al.* 1997). The aligned sequences were processed by MEGA 6.0 (Tamura *et al.* 2013) for analyzing the DNA sequence compositions and calculating the pairwise genetic distance based on the Kimura-2-parameter model (Kimura 1980). The COI sequences of new species and known species obtained in this study were submitted to GenBank with the following accession numbers: *Holorusia basiflava* (KY861847), *Tipula (Formotipula) maolana* (KY861854), *Tipula (Formotipula) holoserica* (KY861856), *Pselliophora guangxiensis* (MF095105), *Pselliophora xanthopimplina* (MF095106), *Indotipula jinxiuensis* sp. nov. (MF095107).

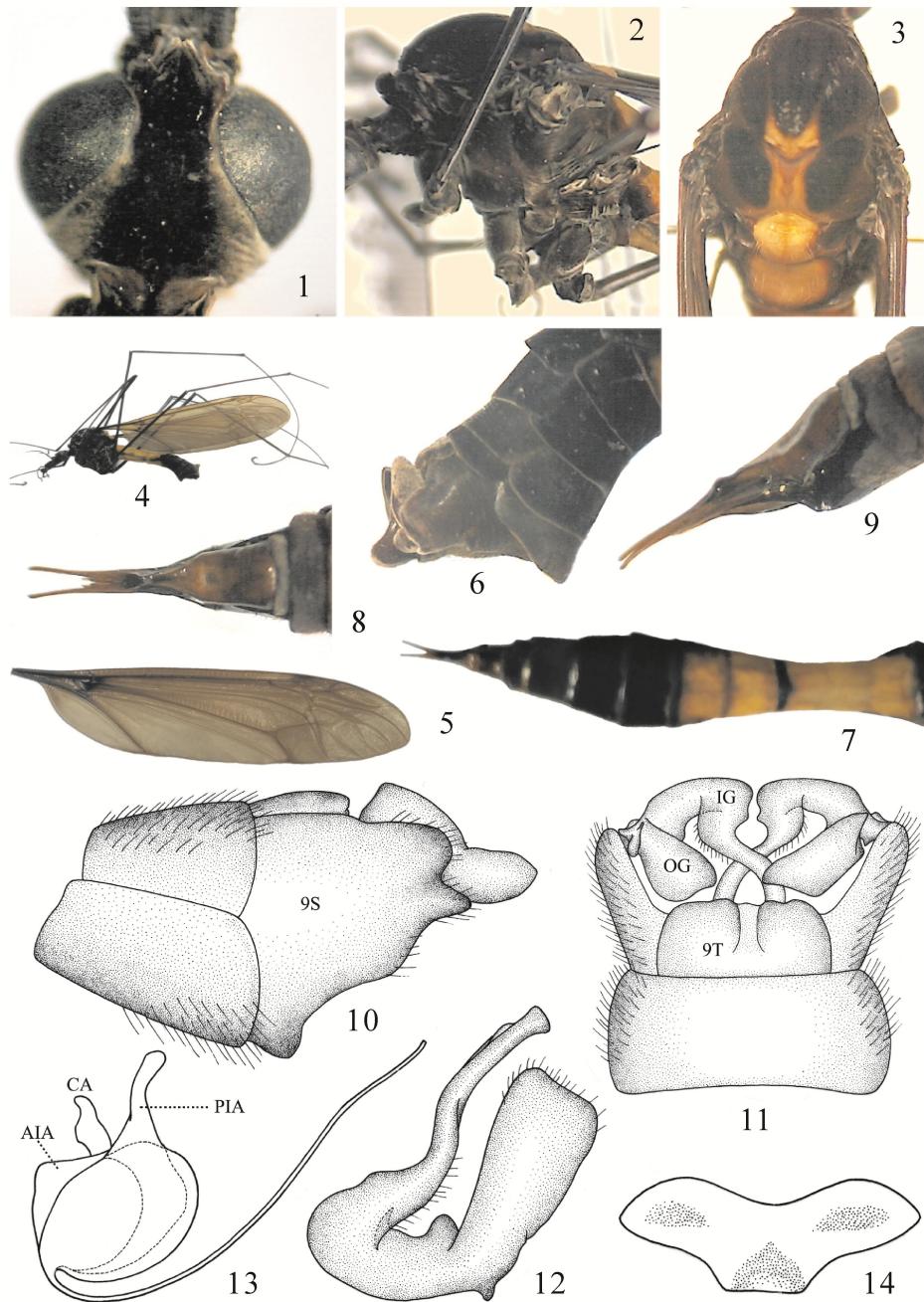
Taxonomy

1. *Holorusia basiflava* Yang & Yang, 1993 (Figs. 1–14)

Holorusia basiflava Yang & Yang, 1993b, 9: 59.

Diagnosis. Generally brown. Prescutum with three black stripes. Hind margin of prescutum, median regions of scutum, scutellum, postnotum and first three abdominal segments variegated by yellow. Wing light brown, stigma darker than ground color. Tergite

nine broadened, hind margin slightly concaved; inner gonostylus narrowed, outer gonostylus slightly curved, broadened apically.



Figures 1–14. *Holorusia basiflava*. 1. Head, dorsal view; 2. Thorax, lateral view; 3. Thorax, dorsal view; 4. Habitus, lateral view; 5. Wing; 6, 10. Hypopygium, lateral views; 7. Abdomen (female), dorsal view; 8. Ovipositor, dorsal view; 9. Ovipositor, lateral view; 11. Hypopygium, dorsal view; 12. Inner gonostylus and outer gonostylus; 13. Semen pump, lateral view; 14. Compressor apodeme, dorsal view.

Redescription. Male. Body length 25.0–27.0 mm, wing 30.0–32.0 mm, antenna 5.0–5.2 mm.

Head. Rostrum brown, nasus lacking. Antennae brown, scape elongated, narrowed basally and gradually widening to apex, pedicel very short, flagellum 12-segmented, first flagellomere longest, slightly longer than scape, the remainder progressively shortening, fourth to eighth flagellomere expanded medially. Palpi brown. Head brown, occiput without mark (Fig. 1).

Thorax. Pronotum wholly brown (Fig. 3). Prescutum brown with three black stripes; the median stripe narrowed basally and gradually widened, rounded apically; the lateral stripes oval, nearly half of length of median stripe; hind margin of prescutum yellow (Fig. 3). Scutum yellow, each lobe with a black stripe (Fig. 3). Scutellum brown with a big yellow spot medially (Fig. 3). Postnotum brown with a yellow spot medially (Fig. 3). Pleura brown without dorsal-longitudinal stripe (Figs. 2, 4). Halteres wholly brown. Legs wholly dark brown (Fig. 4). Wing light brown, stigma darker than ground color; veins brown (Figs. 4, 5). Rs relatively short, cell m1 petiolate, discal cell narrowed, subequal in length to cell m1 (Figs. 4, 5).

Abdomen with first three segments yellow, tergites of which suffused with narrowed yellow margins on laterals, generally yellow, rest of segments and hypopygium wholly brown (Fig. 4). Hypopygium broadened (Figs. 6, 10). Tergite nine broadened with hind margin slightly concaved (Fig. 11). Sternite nine with a deep V-shaped notch medially. Inner gonostylus narrowed, S-shaped (Fig. 12); outer gonostylus flake-like, slightly curved, broadened apically (Fig. 12).

Semen pump with compressor apodeme V-shaped (Fig. 14). Posterior immovable apodeme curved dorsally, widened basally, distinctly longer than compressor apodeme, forming an 80° angle with compressor apodeme (Fig. 13). Anterior immovable apodeme with arms broad, forming an obtuse angular flake (Fig. 13). Aedeagus slender, almost 2.0 times longer than semen pump, subequal in thickness throughout, truncate apically (Fig. 13).

Female. Body length 29.0 mm, wing 31.0 mm, antenna 5.2 mm.

The general coloration of head, thorax and abdomen similar to that of male (Fig. 7). Ovipositor brown (Figs. 8, 9). Cerci long, acinacifoliate (Figs. 8, 9). Hypovalva simple, extending to three fifths length of cerci (Figs. 8, 9).

Specimens examined. 11♂1♀, **China**, Hekou, Dayaoshan National Nature Reserve, 24°09'N, 110°06'E, 14-V-2016, coll. Qiulei MEN. GenBank accession number: KY861847.

Distribution. China (Guangxi).

2. *Pselliophora guangxiensis* Yang & Yang, 1988 (Figs. 15–33)

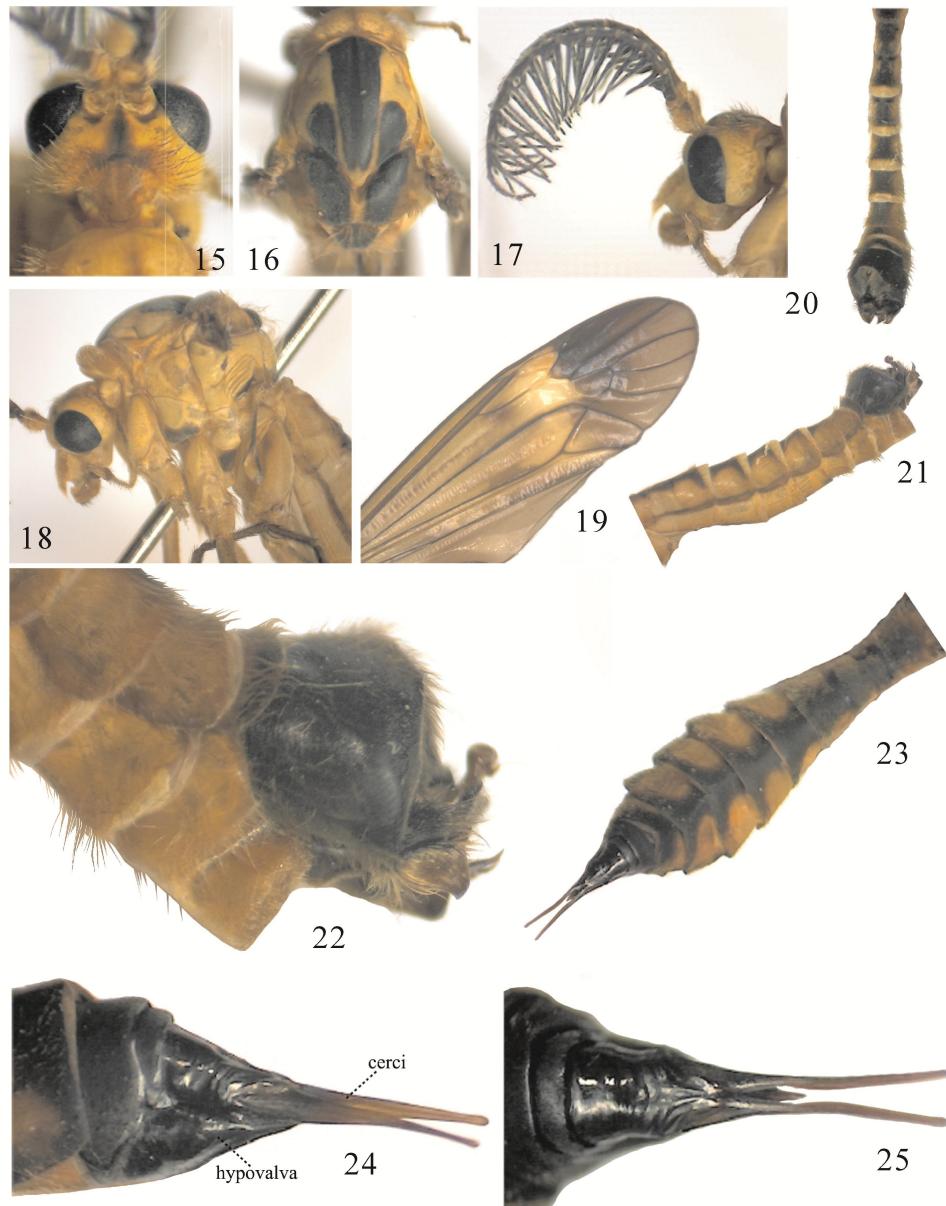
Pselliophora guangxiensis Yang & Yang, 1988, 10: 5.

Diagnosis. General yellow. Prescutum with three black stripes, the median one with a black mid-line. Wing yellowish-brown, apical one third of wing dark brown. Abdominal tergites with broad median stripe and narrow lateral stripes. Tergite nine broadened and black, medially with a V-shaped notch, lateral angles terminated into a sharp process, a small triangular process generated from the middle of notch.

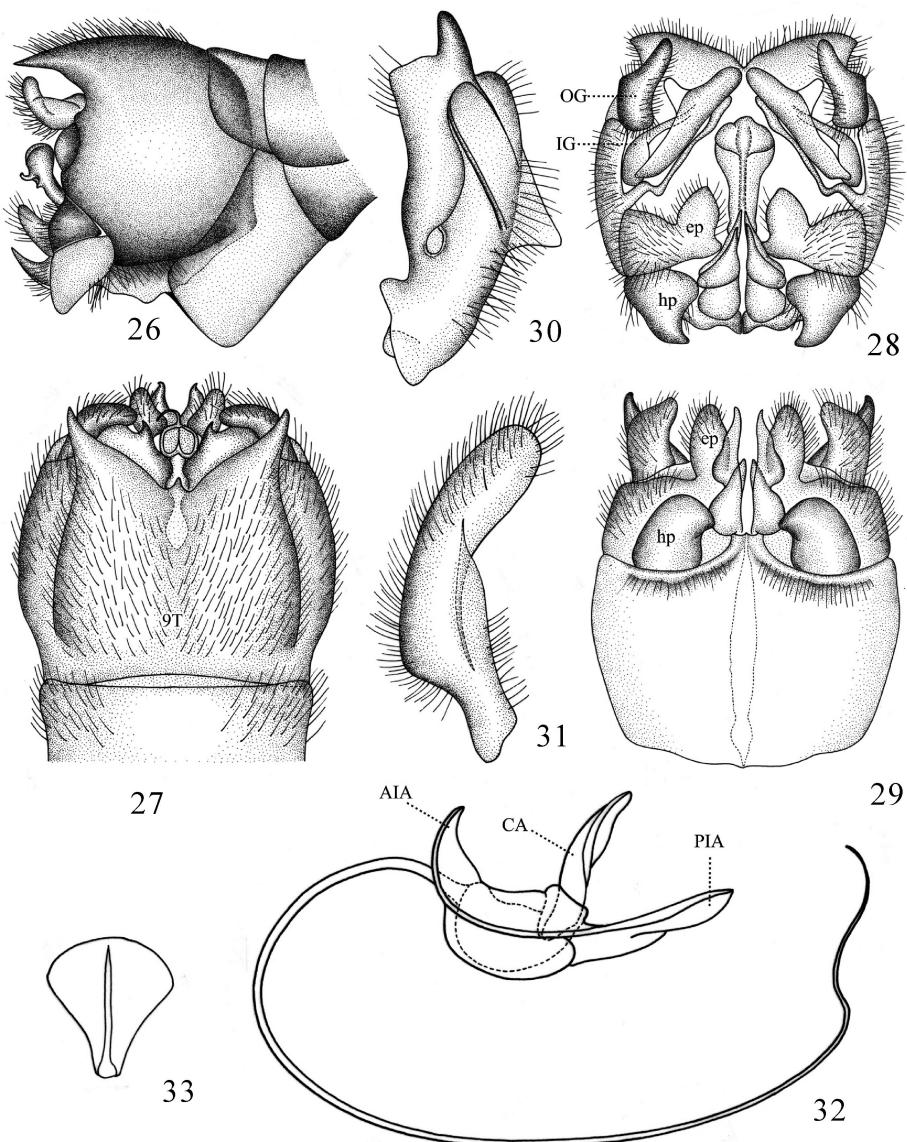
Redescription. Male. Body length 18.0–21.0 mm, wing 17.0–18.0 mm, antenna 5.3–5.5 mm.

Head. Rostrum yellow with short nasus distinct, densely covered with brown setae (Fig. 15). Antennae bipectinate, scape yellow, elongated, narrowed basally and gradually widened

to apex, pedicel yellow, very short, flagellum black, 10-segmented, first flagellomere with only one branch, second to tenth flagellomere with two pairs of branches generally subequal in length (Fig. 17). Palpi yellow. Head yellow, occiput with a subtriangular brown mark (Figs. 15, 17).



Figures 15–25. *Pselliophora guangxiensis*. 15. Head, dorsal view; 16. Thorax, dorsal view; 17. Head, lateral view; 18. Thorax, lateral view; 19. Wing; 20. Abdomen (male), dorsal view; 21. Abdomen (male), lateral view; 22. Hypopygium, lateral view; 23. Abdomen (female), dorsal view; 24. Ovipositor, lateral view; 25. Ovipositor, dorsal view.



Figures 26–33. *Pselliophora guangxiensis*. 26. Hypopygium, lateral view; 27. Hypopygium, dorsal view; 28. Hypopygium, caudal view; 29. Hypopygium, dorsal view; 30. Inner gonostylus, inner view; 31. Outer gonostylus, outer view; 32. Semen pump, lateral view; 33. Compressor apodeme, dorsal view.

Thorax. Pronotum yellow with a light brown spot in middle (Fig. 16). Prescutum yellow with three black stripes; the median stripe narrowed basally and gradually widened apically, with a dark median line; the lateral stripes oval, nearly half of length of median stripe. Scutum yellow, each lobe with a black stripe (Fig. 16). Scutellum yellow with a brown spot medially (Fig. 16). Postnotum yellow with a brown spot near the hind margin (Fig. 16). Pleura yellow without dorsal-longitudinal stripe (Fig. 18). Halteres yellow. Legs with coxae and trochanters yellow, femora and tibiae brown, the femora with brown tips; anterior and median femur with

a white spot near the base on outer side, posterior femur with a white ring near the base; tibiae and tarsi dark brown. Wing yellowish-brown, apical one third of wing dark brown, stigma yellow (Fig. 19); veins brown. Rs relatively short, cell m₁ sessile, discal cell broad, distinctly shorter than cell m₁ (Fig. 19).

Abdomen generally yellow, tergites one to eight with broad median stripes and narrowed lateral stripes, tergite nine wholly black (Figs. 20, 21). Sternites yellow with indistinct brown spots medially. Hypopygium compressed and broadened (Figs. 22, 26). Tergite nine broadened, medially with a V-shaped notch, lateral angles terminated into a sharp process, a small triangular process generated from the middle of notch (Figs. 26, 27). A pair of horn-shaped (hp) processes on the hind margin of tergite nine, above it equipped with a pair of ear-shaped lobes (ep), between these appendages with paired lobes with two layers and acute apically (Figs. 28, 29). Sternite nine with posterior margin straight, smaller than tergite nine (Fig. 26). Outer gonostylus curved, widened basally, rounded apically (Fig. 30). Inner gonostylus slightly curved, widened apically with a black beak at apex, medially with a transparent sclerotized lobe on lateral, basal one third with a node on inner side (Fig. 31).

Semen pump with compressor apodeme (CA) fan-shaped (Fig. 33). Posterior immovable apodeme (PIA) straight, widened basally, slightly longer than compressor apodeme, forming a 75° angle with compressor apodeme (Fig. 32). Anterior immovable apodeme (AIA) with arms broad, gradually narrowed to apex (Fig. 32). Aedeagus slender, at least 3.5 times longer than semen pump, thickened basally and gradually narrowed to apex, very acute apically (Fig. 32).

Female. Body length 21.0–23.0 mm, wing 21.0–23.0 mm, antenna 2.2–2.4 mm.

Antennae relatively short, scape yellow, elongated, narrowed basally and gradually widened to apex, pedicel yellow, very short, flagellum yellow, 10-segmented, moniliform. Abdomen generally yellow, tergites one to seven with broad median stripes and narrowed lateral stripes, tergite nine and ten wholly black (Fig. 23). Sternites yellow with distinct brown stripe medially. Ovipositor black (Figs. 24, 25). Cerci long, acinacifoliate, surpassing the end of hypovalva (Figs. 24, 25). Hypovalva simple, extending to nearly half length of cerci (Figs. 24, 25).

Specimens examined. 4♂4♀, **China**, Lianhuashan Mountain, Dayaoshan National Nature Reserve, 24°09'N, 110°06'E, 16-V-2016, coll. Qiulei MEN. 2♂, Lianhuashan Mountain, Dayaoshan National Nature Reserve, 24°09'N, 110°06'E, 02-V-2015, coll. Guoxi XUE. GenBank accession number: MF095105.

Distribution. China (Guangxi).

3. *Pselliophora xanthopimplina* Enderlein, 1921 (Figs. 34–42)

Pselliophora xanthopimplina Enderlein, 1921, 52: 224; *Pselliophora quadrivittata* Edwards, 1921, 7: 377; Alexander, 1940, 19: 122; Savchenko, 1973, 2: 209; Li *et al.*, 2016, 8: 18.

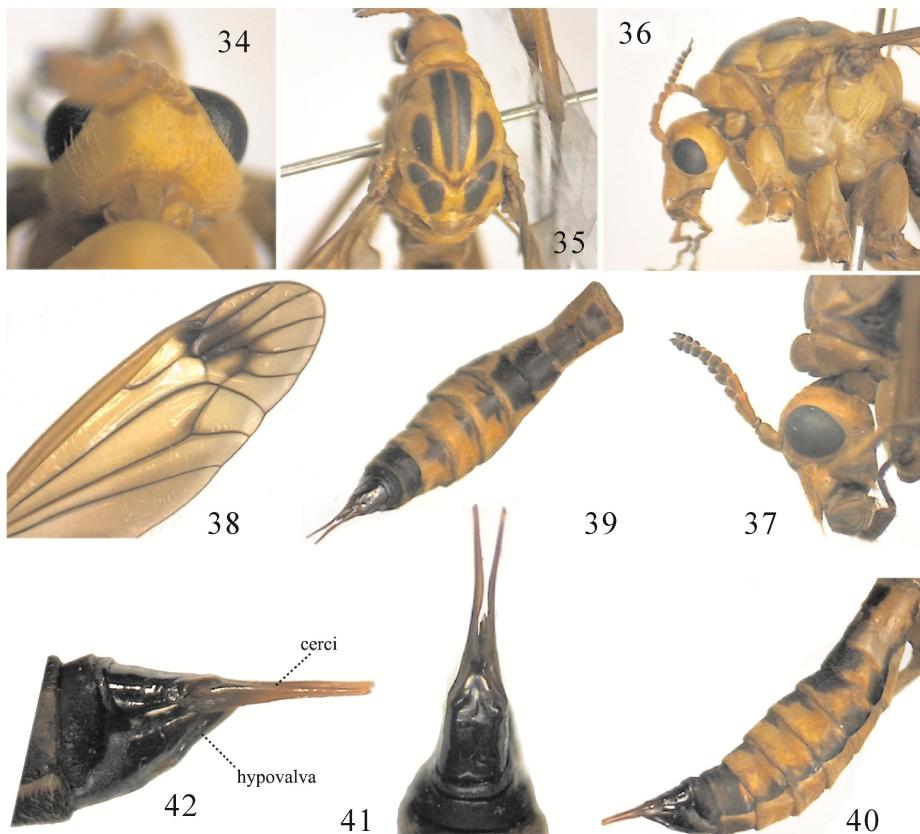
Female. Body length 19.0–22.0 mm, wing 19.0–22.0 mm, antenna 2.2–2.5 mm.

The general coloration of head, thorax and abdomen similar to that of male (Figs. 34–38).

Antennae relatively short, scape yellow, elongated, narrowed basally and gradually widened to apex, pedicel yellow, very short, flagellum yellow on inner side but light brown on outer side, 10-segmented, moniliform (Fig. 37).

Abdomen generally yellow, tergites one to four with broad median stripes and narrowed lateral stripes, tergite five to seven with median stripe relatively narrow or sometimes lacking,

tergites eight to ten and ovipositor wholly black (Fig. 39). Sternites yellow with indistinct brown stripe medially (Fig. 40). Ovipositor black (Figs. 41, 42). Cerci long, acinacifoliate, surpassing the end of hypovalva (Figs. 41, 42). Hypovalva simple, extending to nearly half length of cerci (Figs. 41, 42).



Figures 34–42. *Pselliophora xanthopimplina*. 34. Head, dorsal view; 35. Thorax, dorsal view; 36. Thorax, lateral view; 37. Head, lateral view; 38. Wing; 39. Abdomen (female), dorsal view; 40. Abdomen (female), lateral view; 41. Ovipositor, dorsal view; 42. Ovipositor, lateral view.

Specimens examined. 7♀, **China**, Lianhuashan Mountain, Dayaoshan National Nature Reserve, 24°09'N, 110°06'E, 16-V-2016, coll. Qiulei MEN. GenBank accession number: MF095106.

Distribution. China (New record: Guangxi; Anhui, Zhejiang, Fujian, Sichuan, Guangdong).

4. *Tipula (Formotipula) holoserica* (Matsumura, 1916)

Formotipula holoserica Matsumura, 1916, 2: 456.

Tipula rufomedia Edwards, 1916, 18: 259. Synonymized by Alexander, 1920, 13: 264.

Tipula nigrorubra Riedel, 1917, 82: 115. Synonymized by Alexander, 1920, 13: 264.

Specimens examined. 5♂, **China**, Lianhuashan Mountain, Dayaoshan National Nature Reserve, 24°09'N, 110°06'E, 15-V-2016, coll. Qiulei MEN. GenBank accession number:

KY861856.

Distribution. China (New record: Guangxi; Taiwan).

5. *Tipula (Formotipula) maolana* Li, Yang & Chen, 2013

Tipula (Formotipula) maolana Li, Yang & Chen, 2013, 35: 198.

Specimen examined. 1♂, **China**, Lianhuashan Mountain, Dayaoshan National Nature Reserve, 24°09'N, 110°06'E, 14-V-2016, coll. Qiulei MEN. GenBank accession number: KY861854.

Distribution. China (New record: Guangxi; Guizhou).

6. *Indotipula jinxiensis* sp. nov. (Figs. 43–55)

Diagnosis. Generally stramineous. Prescutum stramineous without distinct stripes. Wing light brown, stigma dark brown. Abdominal tergites and hypopygium brown. Tergite nine with two rounded lobes on hind margin.

Redescription. Male. Body length 8.4–8.6 mm, wing 14.0–14.2 mm, antenna 5.1–5.3 mm.

Head. Rostrum yellow with nasus distinct. Antennae with scape yellow, elongated, cylindrical, pedicel yellow, very short, flagellum dark brown, flagellomere generally subequal in length. Palpi light brown. Head yellow, occiput with median region slightly darker in coloration (Fig. 43).

Thorax. Pronotum stramineous (Figs. 44, 45). Prescutum stramineous without distinct stripes (Fig. 44). Scutum, scutellum and postnotum stramineous (Fig. 44). Pleura yellow, without dorsal-longitudinal stripe (Fig. 45). Halteres light brown. Legs with coxae and trochanters yellow, femora light brown with dark tips, the femora with brown tips; tibiae and tarsi dark brown. Wing light brown, stigma dark brown; veins brown. Rs relatively short, cell m1 short petiolate, discal cell broad, distinctly shorter than half of cell m1 (Fig. 46).

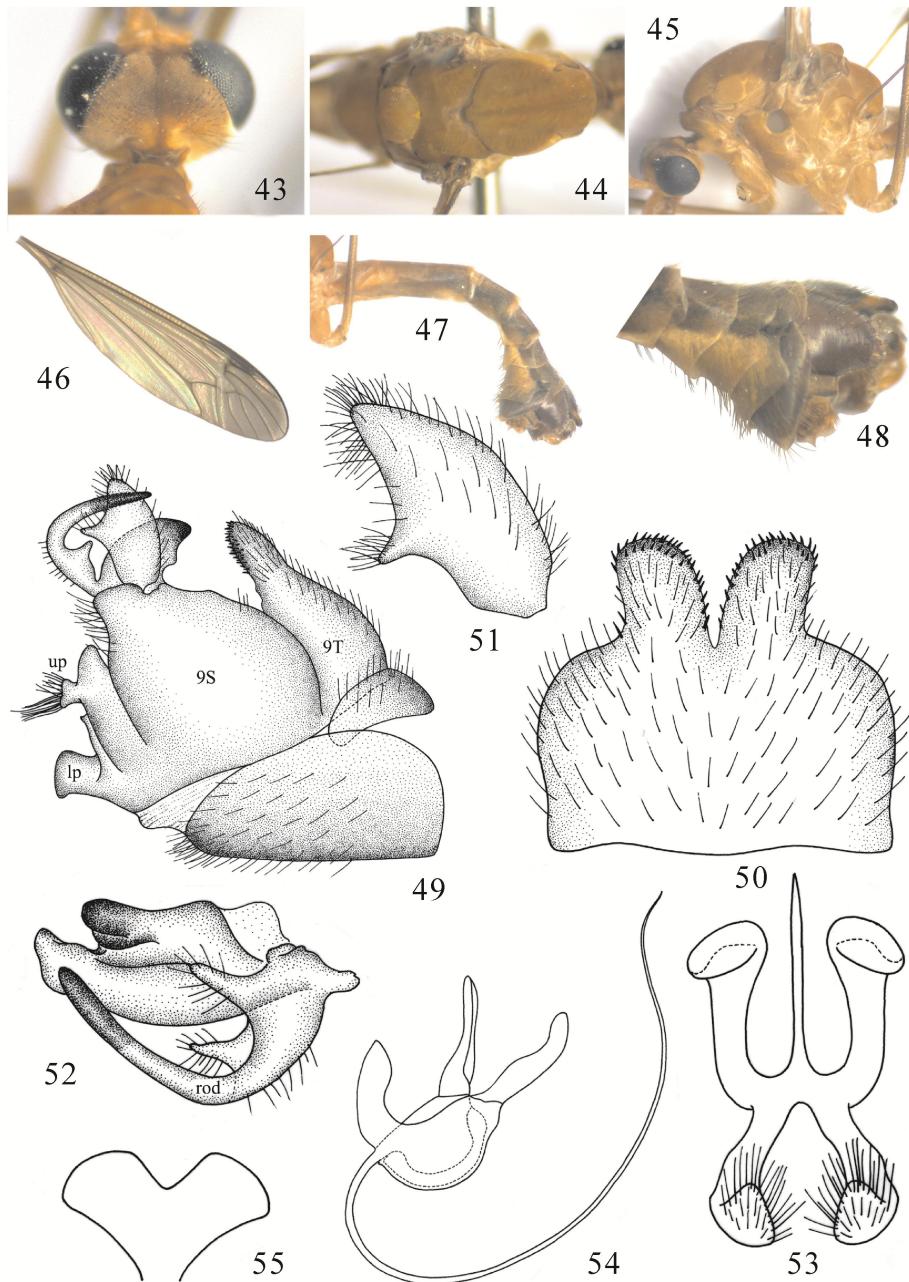
Abdomen with tergites brown, sternites one to seven yellow, sternite eight brown (Fig. 47). Hypopygium brown, broadened (Fig. 48). Tergite nine produced into two flattened and rounded lobes on hind margin which are separated by a V-shaped notch, many black setae scattered on apex and inner margins of lobes (Figs. 49, 50). Sternite nine broad, two processes pointed caudally in lateral view, the upper (up) one truncate, densely covered with long hairs, the lower one (lp) bigger than upper one, also truncate apically (Fig. 49). Outer gonostylus narrowed apically, with an angular process on inner margin medially (Fig. 51). Inner gonostylus includes two parts: outer part with three processes, two small on base, an elongated rod on apical; inner part terminated into two beaks (Fig. 52). Aedeagal guide with four arms, the upper two longer than lower two which extended to outside of genital chamber, forming the upper process of sternite nine (Fig. 53).

Semen pump with compressor apodeme V-shaped (Fig. 55). Posterior immovable apodeme curved dorsally, subequal in length with compressor apodeme, forming a 75° angle with compressor apodeme (Fig. 54). Anterior immovable apodeme with arms broad, slightly curved caudally (Fig. 54). Aedeagus slender, at least 2.5 times longer than semen pump, thickened basally and gradually narrowed to apex, very acute apically (Fig. 54).

Holotype. ♂, **China**, Lianhuashan Mountain, Dayaoshan National Nature Reserve, 24°09'N, 110°06'E, 15-V-2015, coll. Qiulei MEN. **Paratypes.** 4♂, same data as holotype. GenBank accession number: MF095107.

Distribution. China (Guangxi).

Etymology. The specific epithet is a noun ‘jinxiu’ with Latin suffix ‘ensis’, referring to the type locality (Jinxiu county) of the new species.



Figures 43–55. *Indotipula jinxiuensis* sp. nov. 43. Head, dorsal view; 44. Thorax, dorsal view; 45. Thorax, lateral view; 46. Wing; 47. Abdomen (male), lateral view; 48, 49. Hypopygium, lateral views; 50. Tergite nine, dorsal view; 51. Outer gonostylus, lateral view; 52. Inner gonostylus, lateral view; 53. Aedeagal guide, dorsal view; 54. Semen pump, lateral view; 55. Compressor apodeme, dorsal view.

Remarks. This new species is placed in the genus *Indotipula* because of its tergite nine having two lobes, as well as the morphology of the wings and body. This new species is similar to a Philippine species, *Indotipula latilobata* (Alexander, 1932), in body color and the structure of tergite nine; it can be easily distinguished from the latter by the outer gonostylus without lobule basally and inner gonostylus without setae along outer margin (outer gonostylus with a glabrous triangular lobule basally and inner gonostylus with seven to nine flattened setae along outer margin in *I. latilobata* as described in Alexander (1932).

COI Sequences Analysis

Variable sites from partial *COI* gene sequences for crane fly species in present study are shown in Fig. 56. There are no gaps in the sequences of 648 sites, which include 154 variable sites and 494 conserved sites. Of 154 variable sites, 88 were parsimony informative sites and 66 were singleton sites. The average nucleotide compositions of guanine, adenine, thymine, and cytosine were 16.9%, 29.0%, 37.9%, and 16.9%. The pairwise distances among the six species in this study ranged from 0.058 to 0.158 (Table 1). The pairwise genetic distances between *P. guanxiensis* and *P. xanthopimplina* as well as between *T. (F.) holoserica* and *T. (F.) maolana* were 0.076 and 0.058 respectively; this is lower than the comparison values between genera (ranging from 0.132 to 0.157) (Table 2).

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1111111111 1111111111 2222222222 2222222222 2233333333 3333333333 3333333333 3344444444
11123334 4555567789 0011233445 6788888999 0123334445 5555677889 9900111122 2222334445 5566777888 9900001122
4746951246 9025843627 3925109257 3612478036 8492592470 3469214392 5814036925 6789140691 2527679358 1704692547
P. guanxiensis GAGTAAAGAT ATATTTTCT ATTTTAAAT TGTTAAAGT ATAATTTACT TCTTTGCTTA TGATTCTCA AGAGAATCAC AATATCTAA AATTAATATT
P. xanthopimplina ...A.....GCA.AT. .AC..C.A. ....G..C ..C..G. ....G. ....C.C.G .A..A.T. ....CA. ....T ..A. ....T
I. jinxuensis sp. n. A.A.GTT..TA ...C.ACG. ..C..C.G ..C..CT.A T..T..CCAA ....T.A. G.TAA..C.T TCT.C..TT ..TCGCTA..G .TA...TCC
H. basiflava AT.....TA GCT..A..T. ....A..C..G .....A. T.GT..CCT. ....CATAG AA..A.T. ..TC..TCCT.. T..ATA..T TTA..TC...
T. (F.) holoserica ...A..ATA .....TA T.....T.A. ....CT.CCAA C..TCAA..T. ATAA..TT... .AC..A..CTT TCT.C..A.G ....A..CTT ..TA..A..A
T. (F.) maolana ....A..ATA ..CT.....A CACCT.CAA C..GT.A..T. ATAA..TA.C. AAT..A.T TCTTT..A.G ....C..CTT ..TACT.A..A
4444444444 4555555555 5555555555 5555666666 6666]
3344556679 9000001111 1122222334 4555677778 8899011111 2234]
3658140650 3256891245 7803469281 4046212470 6925403679 2840]
P. guanxiensis ATATATATCA ATTGTCCTC TCTCTATAA TTAAATTATT TCCTATTCT TTTT
P. xanthopimplina ...AC..CA. ....TA..GT A..CA..CTAA. ....CT..A ..T..AGCA. ....
I. jinxuensis sp. n. ..CGA..ATCAT ..A..A..TA. ....TAA..TA..G ..AC..C..CA ..TT..G. ....
H. basiflava T....AT..AT TAA..TATAA A..ACTATC.. ....ACT..A ..TTC..CA..TA..C..C.
T. (F.) holoserica ...C..AT... ..TA..ACTA... ....TA..AAT AA..TC..A..CT.....C..C ...
T. (F.) maolana ....AT..TT TAAA..TA... ....TATAA. ..A.....A..CTT..... .C..C

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Figure 56. Variable sites from mitochondrial *COI* gene sequence alignment. Numbers arrayed vertically represent the positions of the nucleotides. Matched sites are marked with dots.

Table 1. Pairwise genetic distances for *COI* gene sequences among species examined in present study

Species	gua	xan	jin	bas	hol	mao
gua						
xan	0.076					
jin	0.130	0.158				
bas	0.130	0.135	0.139			
hol	0.115	0.142	0.156	0.148		
mao	0.128	0.156	0.158	0.130	0.058	

Abbreviation: *gua*, *P. guanxiensis*; *xan*, *P. xanthopimplina*; *jin*, *I. jinxuensis* sp. nov.; *bas*, *H. basiflava*; *hol*, *T. (F.) holoserica*; *mao*, *T. (F.) maolana*.

Table 2. Pairwise genetic distances for *COI* gene sequences among genera examined in present study

Genus	<i>Holorusia</i>	<i>Indotipula</i>	<i>Pselliophora</i>	<i>Tipula (Formotipula)</i>
<i>Holorusia</i>				
<i>Indotipula</i>	0.132			
<i>Pselliophora</i>	0.139	0.144		
<i>Tipula (Formotipula)</i>	0.132	0.157	0.135	

Key to males of *Indotipula* species from China

1. Sternite nine terminated into an acute apex..... *I. demarcata*
- . Sternite nine not terminated into an acute apex..... 2
2. Sternite nine with a hairy process pointed caudally..... 3
- . Sternite without such process..... 4
3. Tergite nine with a pair of spinulous lobes..... *I. suensoni*
- . Tergite nine with a pair of rounded lobes..... *I. jinxiuensis* sp. nov.
4. Inner gonostylus and outer gonostylus relatively narrow..... *I. yamata subyamata*
- . Inner gonostylus and outer gonostylus relatively broad..... *I. yamata yamata*

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